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PRINCIPAL INVESTIGATOR: Mauricio Rojas

CONTRACTING ORGANIZATION: University of Pittsburgh
Pittsburgh, PA 15213

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E-Mail: rojasm@upmc.edu

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University of Pittsburgh. 3520 Fifth Ave.
Pittsburgh PA 15213-3320

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Transfer of injured service members from the Level 3 combat support hospital to level 4 and 5 medical facilities increase their chance of survival from devastating injuries. Aeromedical evacuation of patients with Acute Respiratory Distress Syndrome (ARDS) is sometimes beyond the possibilities because of limitations providing ventilator support in flight with a possible further deterioration in patient status. Cell based therapy with adult bone marrow-derived mesenchymal stromal cells (MSC) in experimental models of ARDS data suggest that administered allogeneic B-MSCs can mitigate hypoxemia and promote recovery. However, it is unknown how this new form of therapy can be used adjunct to current supportive measures for lung failure. Our objective is to complete a series of preclinical studies in large animal models using extracorporeal membrane oxygenation (ECMO) alone or in combination with MSC in sheep and pigs with ARDS. Our group had completed the first 19 experiments in which we demonstrated that 3.5 ug/kg of LPS infused i.v. to a sheep induces lung injury equivalent to a moderated ARDS. In a second group of studies sheep in which respiratory support was providing by a low flow-low pressure ECMO (ALung) partially rescued the animals returned the parameters of respiratory function to normal values. It is our goal to now use Alung in combination of MSCs to potentiate their protective effect.

15. SUBJECT TERMS
LPS induced ARDS, Alung, lung injury

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Table of Contents

Introduction.

Transfer of injured service members from the Level 3 combat support hospital to level 4 and 5 medical facilities increase their chance of survival from devastating injuries. Aeromedical evacuation of patients with Acute Respiratory Distress Syndrome (ARDS) is sometimes beyond the possibilities because of limitations providing ventilator support in flight with a possible further deterioration in patient status. Cell based therapy with adult bone marrow-derived mesenchymal stromal cells (MSC) in experimental models of ARDS has been the focus of intense by investigation. Our previously published data suggest that administration of allogeneic MSCs can mitigate hypoxemia in ARDS and promote recovery. However, it is unknown how this new form of therapy can be used as an adjunct to current supportive measures for lung failure.

Our objective is to complete a series of preclinical studies in large animal models using two different protocols of extracorporeal membrane oxygenation (ECMO) alone or in combination with MSC in sheep. In a separate set of experiments, our collaborators in the USAISR in San Antonio, TX, will be using a pig model of ARDS in combination of a low flow ECMO. Our goal is to use a combination therapy of MSC and ECMO leading to a reduction in invasiveness of mechanical ventilation and inflammatory mediators as well as improvement in oxygenation and functional outcome.

Keywords

Acute respiratory distress syndrome (ARDS), extracorporeal membrane oxygenation (ECMO), Mesenchymal Stromal Cells (MSCs), transport injured service members.

Accomplishments

It is possible to summarize the achievements of each on of the four quarters to evaluate the progress of the project

First Quarter: Completion of all the administrative component. All the protocols required were approved.
Second Quarter: Standardization of the animal preparation, initiation of the control group.
Third Quarter: Completion of the Control group and initiation of the low Flow ECMO (ALung)
Fourth Quarter: Completion of the Group that received LPS and supported with ALung.

I am including the list accomplishments that were reported in each quarter.

-First Quarter:

1. Complete all the administrative requirements to do the studies, including the modifications to the original IACUC Protocol 15034837

2. Coordination of the activities. Our group had 3 meetings with the different members to coordinate all the details of the experiment

3. On January 6 we did our first assay from the control group. All the parameters proposed were measured.

4. During the next two weeks we will be analyzing the data and evaluating the protocols.

-Second Quarter:

1. After completing our first Milestone during the first quarter, during the the following 2 years our goal is to complete a full experimental preparation every week.

2. We are proposing to have five groups each one with different conditions. Completion of each group is going to be considered as a Milestone. We are in the process to complete the second Milestone, the group Control.

-Third Quarter:

2. We are proposing to have five groups each one with different conditions. Completion of each group is going to be considered as a Milestone. We are in the process to complete the second Milestone, the group Control.

For this **Fourth Quarter** these are our main achievements:

We had completed a total of 19 preparations (**Table 1**). After a detailed analysis of the data of each experiments we had decided that 3 animals (experiments 2, 4 and 6) had to be excluded from the final analysis because they had complications, cardiac arrest and atrial fibrillations, during the preparation of the experiment before ARDS was induced. We consider these as complication were no related to the design of the experiment. Other animals had some level of complications, but those were not sufficient to exclude the data.

Since the experiment number 10 we had an anesthesiologist dedicated to the experiment, Tomas Drabek, who is an Associated Professor in the Department of Anesthesiology at the University of Pittsburgh joined our group. The incorporation of Dr Drabek had increased the reproducibility of the experiment. His main goal is to maintain the animal alive with the minimal intervention possible. This had allowed us to compare each one of the interventions.

Table 1

ID	Group	Gender	Weight (Kg)	Notes
S2016-01	LPS	MALE	43	
S2016-02	SALINE	FEMALE	48.2	Cardiac arrest
S2016-03	SALINE	MALE	53.5	Syringe pump issue
S2016-04	LPS	MALE	52	Atrial fibrilation
S2016-05	SALINE	MALE	39	Flipping over
S2016-06	LPS	MALE	31.2	3 Cardiac Arrest in first 30 min.
S2016-07	LPS	FEMALE	38.1	
S2016-08	LPS	FEMALE	36	
S2016-09	PBS+ALUNG	MALE	36	First ALUNG- Standarization
S2016-10	LPS	FEMALE	54	
S2016-11	LPS+ALUNG	FEMALE	46.5	
S2016-12	LPS+ALUNG	MALE	47.5	Tamponade
S2016-13	LPS+ALUNG	FEMALE	50	Cardiogenic Shock 20 min after LPS
S2016-14	LPS+ALUNG	FEMALE	40	Time 5 (Tachyc-Hypot)
S2016-15	PBS+ALUNG	MALE	55.8	
S2016-16	LPS+ALUNG	MALE	49.5	
S2016-17	LPS+ALUNG	FEMALE	51.4	
S2016-18	LPS+ALUNG	FEMALE	48	Cardioverted 3 times
S2016-19	LPS+ALUNG	MALE	51.8	

Another important modification is to have a team meeting every 6-8 weeks. Our group meets for 5-6 hours to discus the project. During the meeting the protocol is revised in detail to define if any modification needs to be implemented and to reaffirm the indications of when the intervention is going to be use. We review the data from each experiment for data analysis and quality control. In case that there is any level of uncertainty of a value, the clinical records are revised. During those meetings we define implementation of small adjustments in the protocol, on sample collection and detailed review of the clinical records. This results and better coordination of the experimental team, an as a consequence the quality of our data had improved.

On the ALung group two animals, numbers 9 and 15, did not received LPS. In the first case, to standardize the preparation, because this was a new protocol in which we were using for first time these type of cannulas, and to be sure that we were not inducing lung injury, at the moment of the surgery, we decided not to use endotoxin.

By doing this we demonstrated that Alung along does not have any negative effect on a normal lung. On experiment 15 we did not observed any injury after LPS. After reviewing all the data of the experiment our conclusion was that the stock of the LPS used was thawed longer than was recommended, reducing the biological activity (**Figure 1**).

Since experiment 11 we are measuring mitochondrial function on the lung and heart tissue (**Figure 2**). The Clark system is allowing us to measure the mitochondrial activity by the oxygen consumption during activation. As a consequence of the LPS-induced injury we observe a decline on mitochondrial function in specific compartments of the heart. Contrary to what was observed in the lung, where ALung was contributing to a small increase in the mitochondrial activity, suggesting a positive effect of ALung on mitochondrial function.

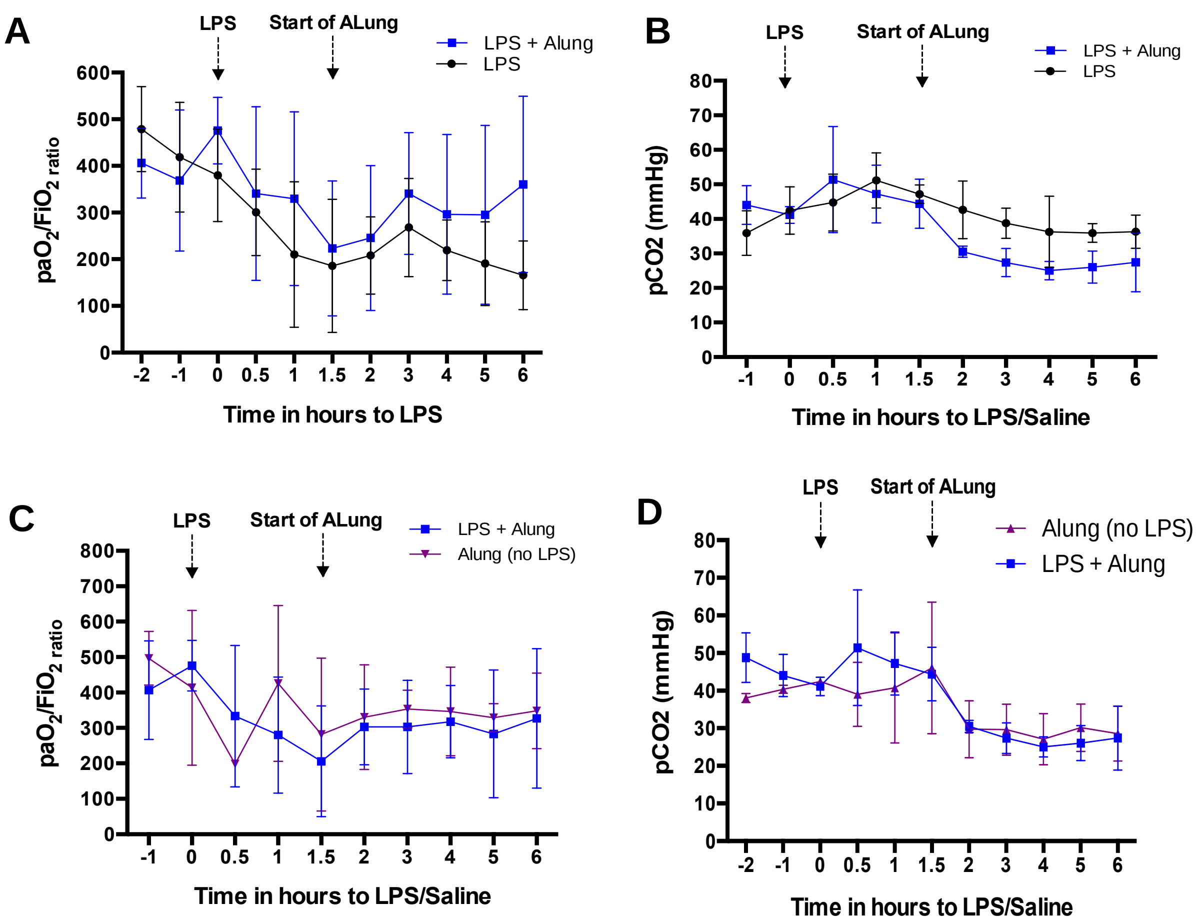

Figure 1. Alung have a moderated level of protection of the lung on sheep with LPS-Induced ARDS**. A-B**. Blood oxygenation and levels of pCO2 are restore to normal levels after 2 hours of Alung, in contrast sheep that did not received any treatment was on moderated to severe ARDS. **C-D** the levels of O2 and CO2 of sheep with ARDS are similar that the normal controls after respiratory support by ALung

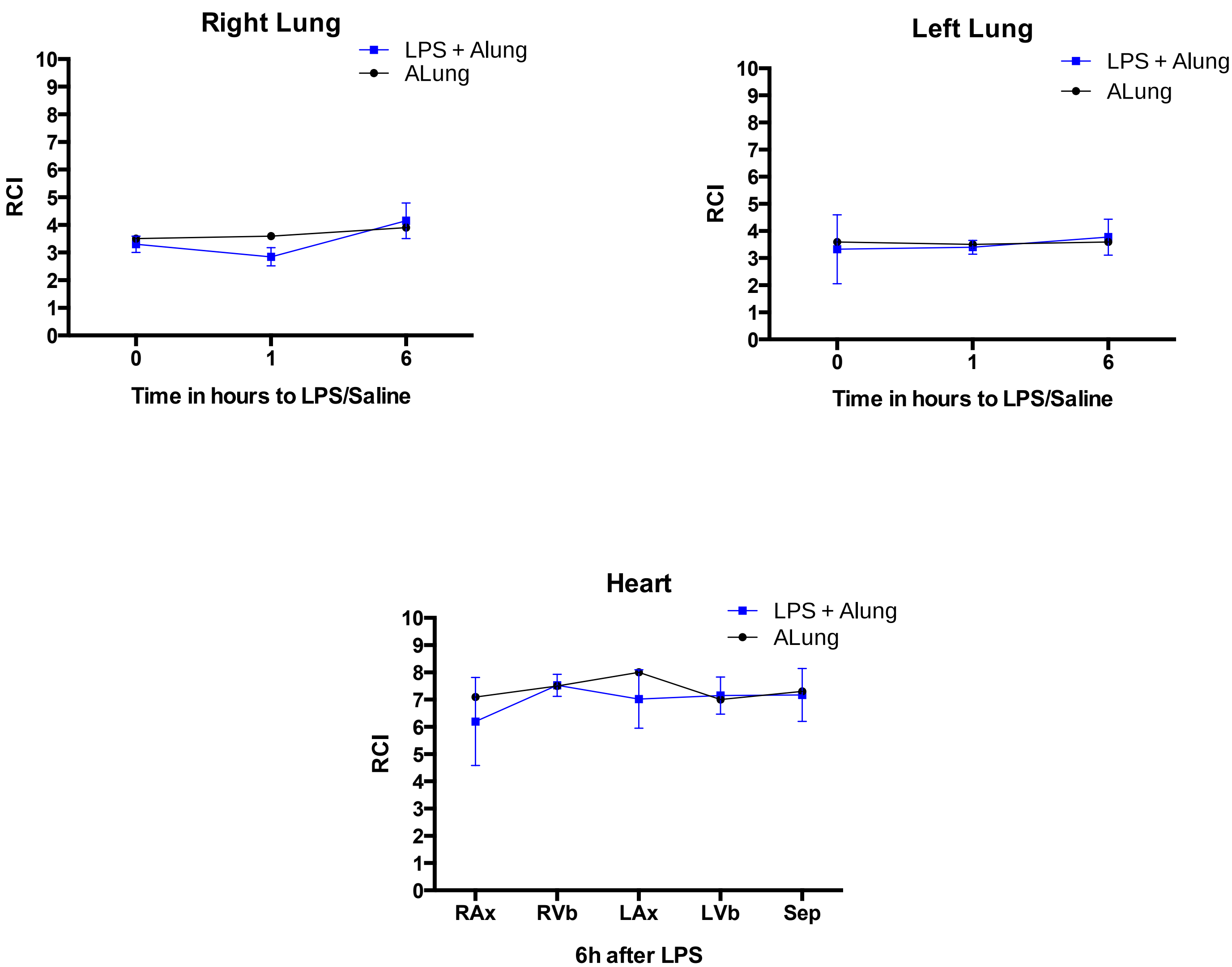

Figure 2. Alung contributes in the homeostasis of the lung mitochondria. Levels of activity of mitochondria are preserved in the right and left lung after LPS-induced ARDS (**A-B**). The respiratory control index (RCI) that is calculated according with the rate of oxygen consumption during phase IV and III of mitochondrial respiration was similar in both groups. **C.** In contrast in the heart in the bases of both ventricles there was a decrease on mitochondrial function.

Impact.

Development of new protocols to treat injured service member sof the military forces can increase the survival and reduce long-term complications. In this initial phase of the study, we had confirmed that by using the proposed animal model we can evaluate the protective effect of any intervention.

As is presented in **Figure 1** the use of Alung, a low invasive, low flow ECMO can improve the conditions of blood oxygenation measured by the ratio PaO_2/FiO_2, we observed an improvement respiratory parameters after 2 hours of respiratory support. This is observation can have a large impact, because blood oxygenation may not be the main indication of this system, the impact of pulmonary rest will result in a reduction on the time and severity of the lung injury. It is our goal for the next phase of experiments to use ALung in combination of MSCs to potentiate the protective effect.

In addition, this is the first study in which heart physiology is evaluated during ARDS; our preliminary data suggest that there is a decrease on mitochondrial function on specific areas of the heart which can result on a decrease of cardiac function. We are analyzing the data of the presion and volume ejected (PV Loops) by the right ventricle during ARDS. This will be the first time that heart is evaluated on this setting of lung injury.

Changes and Problems

As we described previously, some animals have severe complications during the surgical preparation of the animal, which included the placement after an open chest of a central line, intracardiac cannulas in each ventricle and respiratory support. 3 experiments can not be include in the data that is going to be analyzed, we are in the process to complete the statistical analysis, however we anticipate that will be necessary to complete additional experiments to provide the statistical power required for this type of studies.

We do not expect significant changes in the protocol. We only require small adjustments, mostly surgical, when we initiate a new group,

Products

N/A

Participants & Other Collaborating Organizations

Personnel	Role	Percent Effort
Mauricio Rojas Associate Professor Department of Medicine McGowan Institute of Regenrative Medicine University of Pittsburgh	PI	38%
Jonathan D'Cunha Associate Professor of Cardiothoracic Surgery Vice Chair, Research and Education Chief, Division of Lung Transplant/Lung Failure Department of Cardiothoracic Surgery	Surgeon	25%
Ergin Kocyildirim Research Assistant Professor Department of Cardiothoracic Surgery	Surgeon	50%
Tomas Drabek Associate Professor Department of Anesthesiology	Anesthesiologist	5%
Ron Poropatich Executive Director of the Center for Military Medicine Research, Professor of Medicine Division of Pulmonary, Allergy, and Critical Care Medicine University of Pittsburgh	Pulmonologist	5%
Bryan McVerry Assistant Professor of Medicine Associate Director Pulmonary and Critical Care Medicine Fellowship Program Director, Translational Research in Acute Lung Injury	Pulmonologist	5%
John Tedrow Assistant Professor Department of Medicina University of Pittsburgh	Pulmonologist	5%
Nayra Cardenes Instructor Department of Medicine University of Pittsburgh	Coordinator	93%
Diana Alvarez Postdocotral Fellow	Postodoc	50%
Kentaro Nora Postdoctoral Fellow	Perfussionist	42%
Chandler Courfield Technician	Technician	50%

Special Reporting Requirement

Our group is in the process to collect information of a cell counter, which will be use to determine number and viability of the MSCs after they are thawed and prepare for infusion. Measurements will be done in the facility immediately before cells are infused. After finding the most accurate we will request authorization. The cost of the system is not going to affect or limited the proposed experiments, contrary will provide reproducibility and precision on the cell based assays.

Appendices

N/A